

Aetiology Of Diarrhoea In Infants In Khartoum State

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Dedication

To the soul of my Mother

To my father....

To the science and knowledge students

To my wife and son...

To my brothers and sisters

To my friends...

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Abstract

This study was carried out to identify enterobacteria and rotavirus type A in infants suffering from diarrhoea in Khartoum State (Khartoum and Khartoum North) . Sixty samples of diarrhoeal stool were collected during the period from May to June 2006 from 60 infants aged between 4 months to 5 years suffering from diarrhoea .

Isolated bacteria were identified on the basis of their cultural and biochemical characteristics and included the following species :
Escherichia coli and *Salmonella typhi* from 5 specimens , *S. paratyphi* from 5 specimens , *S. typhimurium* from 4 specimens , *Shigella dysenteriae* from one specimen , *Sh. Flexneri* from 2 specimens, *Sh.boydii* from one specimen , *Proteus vulgaris* from 2 specimens , *Klebsiella pneumoniae* from 4 specimens , *K. rhinoschermatis* from 2 specimens and *Pseudomonas aeruginosa* from 6 specimens .

Using direct Enzyme Linked Immuno Sorbant Assay (ELISA) rotavirus type A was detected in five samples associated with *E. coli* and with *Sh. boydii* in one specimen and with *Ps. aeruginosa* in one specimen .

The susceptibility of isolated bacteria to some antibiotics using the multi disk method was variable.

ملخص البحث

هدفت هذه الدراسة إلى التعرف على البكتيريا المعوية وفيروس الروتا من النوع أ عند الأطفال الذين يعانون من حالات الإسهال في ولاية الخرطوم (الخرطوم و الخرطوم بحري). حيث تم جمع 60 عينة برازية من 60 طفل يعانون من الإسهال تتراوح أعمارهم من 4 أشهر - 5 سنوات و في الفترة من مايو إلى يونيو 2006م.

تم عزل و التعرف على أنواع من أجناس البكتيريا المعوية باستخدام الصفات المزرعية و الاختبارات الكيموحيوية. اشتملت على : الاشريشيا القولونية ,سالمونيلا تايفية من 5عينات , سالمونيلا نظير التايفية من 5 عينات , سالمونيلا تافيمرية من 4 عينات , شيجلا الدسنتاريا من عينة واحدة , (شيجلا فليكس من عينتان , شيجلا بويد من عينة واحدة , المتقلبة الاعتيادية من عينتان , كليبسلا الرئوية من 4 عينات , كليبسلا الصلبوم الانفي من عينتان و الزائفة الزنجارية من 6 عينات.

فيروس الروتا النوع أ تم التعرف عليه باستخدام اختبار الاليزا مباشرة.

أظهرت النتائج وجود 5 عينات تحتوي على فيروس الروتا من النوع أ مع وجود بكتيريا أخرى مصاحبة في كل عينة. وهذه البكتيريا تشمل : الاشريشيا القولونية، الشيجلا بويد في عينة واحدة و بكتيريا الزائفة الزنجارية في عينة واحدة.

البكتيريا المعزولة تم إخضاعها لاختبار الحساسية للمضادات الحيوية , حيث أظهرت النتائج وجود تباين في فعالية هذه المضادات الحيوية .

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Chapter one

Introduction

It is estimated that over one billion episodes of acute diarrhoea occur annually in children under 5 years of age in Asia, Africa, and Latin America, resulting in about 5 million deaths. Moreover, the dehydration caused by diarrhoeal diseases accounts for one-third to one-half of infant mortality in many developing countries. The major causes of such diseases are Enterobacteriaceae (Volk and Wheeler, 1984).

The word diarrhoea is derived from the Greek words for (flowing through) . For most persons, diarrhoea means the frequent passage of loose stools (Talley *et al* ., 1994). Diarrhoea is a universal human experience and persons have had acute infectious diarrhoea at some time during their lives (Thielman and Guerrant, 2004)

For clinical purposes, diarrhoea can be classified as either acute (< 4 weeks' duration) or chronic (> 4 weeks' duration). Chronic diarrhoea is further divided into watery, inflammatory, and fatty on the basis of stool characteristics. The value of this classification is that it allows the physician to direct evaluation and management more effectively, because diarrhoeal diseases can be distinguished by the duration of illness and the type of stools produced (Fine and Schiller , 1999).

Most forms of acute diarrhoea (i.e., those lasting less than 4 weeks) are caused by infections and are self-limiting; the majority are caused by viruses (e.g., adenovirus, Norwalk agent, rotavirus), but some are caused by bacteria (e.g., salmonella, shigella, and *Escherichia coli*) and others by protozoa (e.g., giardia and amoebas) (Thielman and

Guerrant ,2004)

Acute diarrhoea is frequent among travellers in whom enterotoxigenic *E. coli* is particularly common (Black, 1986). In practice, most episodes of acute diarrhoea that are assumed to be caused by an infectious agent are treated without the causative agent being identified. Major causes of acute infectious diarrhoea are mainly according to local factors such as availability of clean water and sanitation (Walker and smith, 1993).

The disease course of most viral and bacterial diarrhoeas lasts less than 1 week; therefore, infectious diarrhoea lasting more than 7 days is more likely to be caused by protozoa (Guerrant *et al* ., 2001) .

Pathogenic infections cause diarrhoea by one of four mechanisms: (1) enterotoxins that subvert the regulatory mechanisms of enterocytes, (2) cytotoxins that destroy enterocytes, (3) adherence to the mucosa by organisms (so-called enteroadherent organisms) that alter enterocyte function as a result of physical proximity to the mucosa, and (4) invasion of the mucosa by organisms that provoke an inflammatory response by the immune system. In general, patients with cytotoxin-mediated diarrhoea and those with invasive organisms experience more toxicity and have more abdominal pain than patients with enterotoxin-mediated diarrhoea or enteroadherent infections (Vazquez and Fang, 2000).

Diarrhoeal diseases are noted to be a major cause of morbidity in children bellow five years of age in Sudan. Most cases are due to bacterial infections. The most commonest bacterial pathogens are *E. coli* strains , *Shigella* strains and *Salmonella* strains. Mixed infection with these organisms also occur. Other potential bacterial pathogens are enterococci, *Proteus spp* , and *Pseudomonas aeruginosa* (Erwa , 1969) . Viruses such as rotaviruses and coronaviruses are reported as

primary causes of diarrhoea in infants (Kidd *et al.*,1989).

1.2 Objectives:

- 1/ Identification of important enterobacteria that cause infantile diarrhoea.
- 2/determination of prevalence of rotavirus type A infection in infants and the association of rotavirus type A with significant bacteria.
- 3/ Determination of susceptibility of isolated bacteria to the commonly used antibiotics .

Chapter two

Literature review

Diarrhoea is defined by the World Health Organization (WHO 1990) as 3 or more loose or watery stools (taking the shape of the container) in a 24-hour period.

Diarrhoea is acute if the illness started under 14 days previously, and persistent if the episode has lasted 14 days or more (WHO, 1988). Normal infants who are exclusively breastfed may pass loose, pasty stools frequently. In this group, the definition is usually based on what the mother considers to be diarrhoea (WHO 1990). Infectious diarrhoea is an episode of diarrhoea that is caused by an infectious agent.

Infections and the malnutrition associated with them are responsible for significant proportion of the 13 million deaths among infants and children under 5 years of age worldwide each year. After respiratory infections, diarrhoeal diseases are the most common illnesses and have the greatest negative impact upon the growth of infants and young children. The causes of diarrhoeal diseases have traditionally been ascribed to water supply and sanitation. In attempts to prevent such diseases, efforts by governments and nongovernmental organization have been focused on and sometimes limited to improving water supply and sanitation as well as promoting and protecting breast-feeding (Motarjemi *et al.*, 1993) demonstrated that weaning foods prepared under unhygienic conditions were contaminated with pathogens and thus were a major factor in the cause of diarrhoeal diseases and were associated with malnutrition.

Childhood gastroenteritis remains a common reason for admission to British paediatric units, although the severity of the disease appears to be diminishing in recent years. Two hundred and fifteen infants and children with gastroenteritis admitted consecutively to four paediatric units in South Wales were studied in order to determine the severity of the disease, the organisms isolated, the frequency of complications and the adequacy of management before admission. Viruses were detected in 65, bacteria in 30, and protozoa in 19, with multiple infection found in 11. There was a low incidence of morbidity and complications, but prolonged diarrhoea (post enteritis syndrome) was present in 24(11%) cases and 77(36%) had received inappropriate treatment before admission. Contemporary gastroenteritis is thus a relatively mild disease in the acute phase, but management before admission to hospital is often inadequate, and prolonged diarrhoea may be a feature in considerable number of cases (Jenkins and Ansari, 1990).

The etiology and pathogenesis of persistent diarrhoea is usually multifactorial and sometimes can not be identified . It is necessary to define if an alteration of the enteric microflora is a risk factor that influences the duration of the diarrhoea. This was proven by Garcia *et al.*, (1994) , they studied 30 infants with acute diarrhoea and 30 with persistent diarrhoea. A sample of duodenal content was taken in search for enteric and anaerobic microorganisms and candida. The results were correlated with nutritional status. They found that the presence of bacterial overgrowth of the duodenal microflora was an important factor for the persistence of the diarrhoea.

A total of 492 deaths by diarrhoea in children below 5 years old in rural population of Ethiopia was studied by Shamebo *et al.*, (1991) . They found that the mortality trends was based on population individuals, the study was established in a two year period and it included boys and girls. Although the rates for boys were generally higher especially during infancy. More deaths occurred in the month of April, June, and July, and October and November indicating two peak seasons in both years.

2.1 Enterobacteriaceae:

2.1.1 Definition :

Enterobacteriaceae is a large, heterogeneous group of Gram-negative rods whose natural habitat is the intestinal tract of humans and animals. The family includes many genera e.g., *Escherichia coli*, *Shigella*, *Salmonella*, *Enterobacter*, *Klebsiella*, *Serratia*, *Proteus*, and others. Some enteric organisms, e.g. *Escherichia coli*, are part of the normal flora and incidentally cause disease, while others, e.g., salmonellae and shigellae, are regularly pathogenic for humans (Brooks *et al.*, 1998). Common characters to members of the group are Gram-negative , aerobic and facultative anaerobe and oxidase negative rods. Some attack sugars fermentatively and reduce nitrate to nitrite. These bacteria are easy to grow on simple media and could survive for 10 years on media in tubes simply sealed with paraffin wax. The organisms being easy and safe to handle, are ideal subjects for students, biochemists, geneticists, and even bacteriologists. Laboratory infections do occur but they are rare events. Some strains are human and animal pathogens and produce intestinal infections and food-poisoning. The group has some epidemiological interest and

importance and means have been found to fingerprint the members quite exactly. Only a few are pathogenic but these may cause serious infections, so that the importance of the group has become greatly exaggerated (Cowan and Steel,1975).

Enterobacteriaceae members are either motile with peritrichous flagella or non motile, and grow on MacConkey's bile-salt-lactose medium. Few are catalase- positive. They ferment glucose in peptone water with the production of either acid or acid and gas, and they break down glucose and other carbohydrates both fermentatively under anaerobic conditions and oxidatively under aerobic conditions (Cruickshank *et al.*,1982).

2.1.2 Antigenic Structure :

The enterobacteriaceae have complex antigenic structure. The members are classified by more than 150 different heat-stable somatic **O** (lipopolysaccharide) antigens, more than 100 heat-labile **K** (capsular) antigens, and more than 50 **H** (flagellar) antigens. In *Salmonella typhi*, the capsular antigens are called **Vi** antigens (Brooks *et al.*,1998).

Enterobacteriaceae share the common structure of the Gram-negative cell wall with an inner plasma membrane, a peptidoglycan layer and an outer membrane (Gillespie, 1994).

2.1.2.1 Somatic O Antigens:

The most external part of the cell wall lipopolysaccharide consists of repeating units of polysaccharide. Some **O**-specific polysaccharides contain unique sugars. **O** antigens are resistant to heat and alcohol and usually are detected by bacterial agglutination. Antibodies to **O** antigens are predominantly **IgM**. While each genus of enterobacteriaceae is associated with specific **O** groups, a single organism may carry several **O** antigens. Thus, most shigellae share one or more **O** antigens with *Escherichia* . *E.coli* may cross-react with some providencia, klebsiella,

and salmonella species. Occasionally, **O** antigens may be associated with specific human diseases, e.g. specific **O** types of *E.coli* are found in diarrhoea and in urinary tract infections (Brooks *et al.*, 1998).

O antigens are found in the wall of the bacterial cell. These antigens are used to subgroup enterobacteria such as salmonella and *Escheerishia* species, (Cheesbroug, 2000). This antigen has three main components, lipid **A**, the oligosaccharide core, and the repeating polysaccharide chain. The lipid **A** portion is responsible for the toxicity of lipopolysaccharide (LPS) as it is a potent activator of host macrophages inducing the release of cytokines such as interleukin 1 (IL-1) and tumor necrosis Factor (TNF). These mediators initiate the chain of pathophysiological responses associated with Gram-negative species: fever, hypotension, metabolic acidosis, and platelet activation. The core oligosaccharide contains hexoses and heptoses and a unique sugar residue 2-keto-3-deoxyoctonic acid. The polysaccharide chain is made up of a repeating sugar motif which defines the serological reactivity of the antigen. It is also important in protecting the organism from the effects of serum and phagocytosis (Gillespie, 1994).

2.1.2.2 Capsular K Antigens :

K or surface antigens surround the cell wall and can therefore interfere with **O** antigen grouping. They can be inactivated, however, by heat. The **Vi** antigen of *Salmonella typhi* and the **B** antigen of some strains of *E. coli* are examples of **K** antigens (Cheesbrogh, 2000).

Several species express a polysaccharide capsule, or fimbriae, or an envelope of protein. These mediate adhesion and protect against phagocytosis. Important examples in human disease are **K1** antigen of *E. coli* and the **K88** antigen of *E. coli* (Gillespie, 1994). **K** antigens are external to **O** antigens on some but not all members of Enterobacteriaceae. Some are polysaccharides, including **K** antigens of *E.*

coli; others are proteins. Klebsiellae form large capsules consisting of polysaccharides covering the somatic **O** or **H** antigens and can be identified by capsular swelling with specific antisera. Human infections of the respiratory tract are caused particularly by capsular types 1 and 2 ; those of the urinary tract, by types 8,9,10, and 24 (Brooks *et al.*,1998).

2.1.2.3 Flagellar H Antigens

Most of the Enterobacteriaceae are motile as they possess flagella. The **H** antigen is a protein and therefore heat-labile. Flagellar antigens can be used in the identification of species and serotypes (Gillespie, 1994). **H** antigens are used to identify *Salmonella* serovars within their somatic groups (Cheesbrough, 2000). **H** antigens are located on flagella and are denatured or removed by heat or alcohol. They are preserved by treating motile variants with formalin. Such **H** antigens agglutinate with anti-**H** antibodies, mainly **IgG**. Within a single serotype, flagellar antigens may be present in either or both of two forms, called phase 1 (conventionally designated by lower case letters) and phase 2 (conventionally designated by Arabic numerals). The organism tends to change from one phase to the other, this is called phase variation. **H** antigens on the bacterial surface may interfere with agglutination by anti-**O** antibody (Brooks *et al.*, 1998)

2.1.2.4 Toxin Production :

Toxins play an important part in the pathogenicity of enterobacteriaceae especially those causing enteric disease (Gillespie, 1994). Most Gram-negative bacteria possess complex lipopolysaccharides in their cell walls . These substances, endotoxins, have a variety of pathophysiologic effects. Many Gram-negative enteric bacteria also produce exotoxins of clinical importance (Brooks *et al.*, 1998).

Exotoxins and enterotoxins are produced by *Shigella dysenteriae*

and enterotoxigenic *E. coli* (ETEC). When lysed, enterobacteriaceae members release endotoxins from their cell wall. This is a feature of all Gram-negative rods. (Cheesbrough, 2000).

2.1.3 Pathogenicity of the Enterobacteriaceae:

2.1.3.1 *Escherichia coli*:

E. coli is one of the most important members of the Enterobacteriaceae. Strains are predominant among the aerobic commensal bacteria in the healthy human intestine and are thus an indicator for faecal pollution in water supplies (Collee *et al.*, 1996). At least five types of intestinal infections that differ in their pathogenic mechanisms have been identified:

- 1- Enterotoxigenic *E. coli* (ETEC) is a common cause of traveller's diarrhoea. Process mediated by enterotoxigenic *E. coli* (ETEC) and causes prolonged hypersecretion of chloride ions and water by the intestinal mucosal cells, while inhibiting the reabsorption of sodium.
- 2- Enteropathogenic *E. coli* (EPEC) is an important cause of diarrhoea in infants. It adheres and makes destruction in the epithelial cells.
- 3- Enterohemorrhagic *E. coli* (EHEC) binds to cells in the large intestine, where they produce an exotoxin (verotoxin, or shiga-like toxin), causing severe form of copious, bloody diarrhoea (hemorrhagic colitis) in the absence of mucosal invasion or inflammation.
- 4- Enteroinvasive *E. coli* (EIEC) causes dysentery-like syndrome with fever and bloody stools. It invades tissue and makes destruction of epithelial cells.
- 5- Enteroadherent *E. coli* (EAEC) also causes traveller's diarrhoea, and persistent diarrhoea of young children (Strohl *et al.*, 2001).

A non- seasonal diarrhoeal episodes in the Jordan Valley

occurred over a 2-month period, during which no traditional enteropathogens were detected by the health authority laboratories. A total of 17 diarrhoeal stool specimens obtained from infants, young children and adults were randomly collected to investigate the presence of unusual a etiological agents. Stools were examined for parasites, ova, viruses and cultured for bacterial pathogens. Recognized pathogenic organisms were detected in 8 out of 17 of the diarrhoeatic patients, one patient of whom had a mixed infection with two agents: rotavirus and enteroinvasive *E. coli* (EIEC), mixed infection in diarrhoeal patients were enteropathogenic *E.coli* (EPEC) and enterotoxigenic *E.coli* (ETEC) were found in other cases in diarrhea. *Enteroinvasive E.coli* (*EIEC*) was the most common enteropathogen detected (Meqdam ,*et al* 1997) .

Nine strains of *E. coli* isolated from infants with diarrhoea between 1947 and 1960 in Australia and designated (enteropathogenic) were examined for phenotypic and genetic characters associated with virulence. Each strain belonged to different serotype. All the isolates were historically significant in that they were amongst the first strains of *E. coli* reported to be usually associated with infantile diarrhoea. Five strains possessed the virulence properties of class1 enteropathogenic *E.coli* (EPEC). All these strains were isolated originally from symptomatic children during outbreaks of diarrhoea. Two isolates from sporadic cases of diarrhoea fulfilled the criteria for classification as class 11 enteropathogenic *E. coli* (EPEC). One strain was identified as enteroaggregative *E.coli* (EAggEC) and the other carried no known virulence-associated properties. These findings indicate that most early isolates which were designated (enteropathogenic) were indeed EPEC, as currently defined.(Robin *et al.*, 1993).

Verotoxigenic *E.coli* (VTEC) together with other serogroups including *Escherichia coli* O157, are important enteropathogens in infants and toddlers in Czechoslovakia. As to enteropathogenic serotypes, verotoxin (VT) production was proven most frequently in strains of serogroup O26, O111 and O128. Diseases caused by them were as a rule manifested by febrile watery diarrhoea with mucus in the stool. In two of five infants with *Escherichia coli* O26 :H11 with VT1 production in titers of greater than or equal to 1:512, blood was found associated with diarrhoea. In one infant haemorrhagic colitis due to *Escherichia coli* O157:H was found. Haemolytic uraemic syndrome associated with Verotoxigenic *E.coli* (VTEC) of serogroups O157, O26, O5 and O1 with VT1 and /or VT2 was observed in the five children. The source of infection was contaminated water. Of the five children one died (Bielaszewska *et al.*, 1990)

2.1.3.2 **Salmonella:**

The term salmonellosis is used to describe any infection caused by members of the genus salmonella. On industrial scale, slaughterhouse workers are faced with salmonellosis as an occupational hazard. Because humans can become asymptomatic carriers of salmonella, infected food handlers are also responsible for the spread of these organisms. The main types of infection are:

- 1 Typhoid : The classical enteric typhoid fever caused by *S. typhi* .
- 2 Salmonella gastroenteritis: Gastroenteritis with accompanying diarrhoea is, without doubt, the most common type of salmonella infection.
- 3 Salmonella septicemia : Septicemia caused by salmonella is a fulminating blood infection that does not involve the

gastrointestinal tract. Most cases of septicemia are caused by *S. choleraesuis* and are characterized by lesions throughout the body. Pneumonia, osteomyelitis and meningitis also may result from salmonella infection. *Salmonella* osteomyelitis is especially prevalent in people who have sickle cell anaemia (Volk and Wheeler, 1984). Extraintestinal manifestations of salmonellosis in paediatric patients are found predominantly in infants less than three months of age. Genital involvements are rare complications. Berner *et al*, (1994) described a case of a 10-week-old boy suffering from severe diarrhoea, who was presented with a swelling of the right testicle after six days of illness; *S. enteritidis* was cultured from the intraoperative swab. All cultures from blood, CSF and urine remained sterile.

The public health laboratories in Germany have been isolating *Salmonella* species in general in increasing numbers during the last three years and there was a steep increase in the isolation rate of *S. enteritidis*. Epidemiological analysis indicated the involvement of chicken and eggs as the most frequent sources of the infectious agents (Sander, 1993)

2.1.3.3 Shigella:

Humans appear to be the only natural hosts for shigellae. They become infected following the ingestion of contaminated foods or water. Shigellae remain localized in the gut, and the debilitating effects of shigellosis are largely attributable to the loss of fluids and electrolytes (Volk and Wheeler, 1984).

Shigella dysenteriae (sometimes called shiga's bacillus) excretes a potent neurotoxin and an enterotoxin. The neurotoxin is causes paralysis and death. A toxin that is similar or identical to the shiga toxin has been reported to be produced by both *Sh.*

flexneri and *Sh. sonnei*. The bacilli contain a large plasmid that is, at least in part, responsible for their virulence. Loss of the plasmid results in the inability of the shigellae to penetrate or invade epithelial cells, and passing the plasmid to avirulent strains confers virulence on them .

Sh. sonnei is the species most frequently isolated in the United States of America (Volk and Wheeler, 1984). Adult infections are usually mild and self-limiting and are, therefore, not routinely treated with antibiotics. *Sh. sonnei* can, however, cause severe diarrhoea in newborns. *Sh. flexneri* , *Sh. boydii* and *Sh. dysenteriae* are rarely seen in the United States of America but are the most prevalent species seen in developing nations (Volk and Wheeler, 1984).

Shigellae invade and destroy the mucosa of the large intestine. Infection rarely penetrates to deeper layers of the intestine, and does not lead to Shigella bacteremia. An exotoxin (Shiga toxin) with enterotoxic and cytotoxic properties have been isolated from these organisms, and its toxigenicity may play a secondary role in development of intestinal lesions (Strohl *et al.*, 2001).

Study made of 159 infants (including 30 neonates) suffering from diarrhoea who were admitted to the Diarrhoea Treatment Center in Dacca, Bangladesh revealed that the infections were caused by *Sh. boydii* and *Sh. sonnei* whereas *Sh. dysenteriae* type 1 , was less common in infants than in older children; the proportion of *sh. flexneri* infections was smaller in the two groups (Huskins *et al.*, 1994)

2.1.3.4 Klebsiella:

There are many species of *Kelbsiella* which contain strains of different capsular serotypes as follows: *K. pneumoniae* has 3 serotype ,

K. edwardsii two serotypes (1 and 2), *K. atlantae* has one serotype (type 1); *K. rhinoscleromatis* has one serotype (type 3); *K. Ozaenae* has 4 serotypes (3,4,5 and 6) (Cowan and Steel, 1975). O and R somatic (cell wall) antigens have been recognized in smooth and rough variant strains of *Klebsiellae*. The *Klebsiellae* are differentiated into 72 capsule serotypes by identification of their **K** antigens (Cruickshank *et al.*, 1982).

M- antigen, this term has sometimes been used for the loose-slime polysaccharide antigen that can be demonstrated in bacteria-free culture supernates by precipitation with capsular antiserum. It appears to have the same chemical composition and antigenic character as the **K**- antigen of the same strain.

2.1.3.5 *Proteus*:

Bernard *et al.* (1980) questioned the cause of acute diarrhoea in infants by *Proteus* spp. They stated that organisms are commonly found in soil, sewage and manure and found with some frequency in normal human feces, but often in much increased numbers in individuals receiving antibiotic therapy or during diarrhoeal diseases due to other organisms. *Proteus* species produce infections in human only when the bacteria leave the intestinal tract. Motile strains of *proteus* contain H antigen in addition to the somatic O antigen. Certain strains share specific polysaccharides with some rickettsiae and are agglutinated by sera from patients with rickettsial diseases.

2.1.3.6 *Pseudomonas* :

Pseudomonas species are widespread in nature, inhabiting soil, water, plants, animals and humans. *Ps. aeruginosa* is an important cause of infection, especially in patients with compromised immunity (Abuqaddom *et al.*, 2003).

Bodey *et al* ., (1983) noted that pseudomonal infections can affect every portion of the GI tract. The disease often affects children and adults. It can range from very mild symptoms to severe with significant morbidity and mortality. Epidemics of pseudomonal diarrhoea can occur in nurseries. Infants may be present with irritability, vomiting, diarrhoea, and dehydration. The infection be in the form of enteritis, with patients presenting with prostration, headache, fever, and diarrhoea (Shanghai fever).

2.2 Viruses:

2.2.1 Rotaviruses:

Since their discovery in the 1970s , human rotaviruses have been recognized as the most important cause of acute infectious gastroenteritis among infants and children worldwide. Rotavirus has been found to infect almost all mammalian and avian species tested. In humans, it is the most frequent gastrointestinal pathogen in infants and children less than 2 years of age. In developing countries, the attack rate peaks at 6 months of age, whereas in developed areas of the world the virus is most commonly found among children 6-12 months of age (Wyatt *et al* ., 1979).

Rotaviruses are RNA viruses of the reoviridae family. The name is derived from its characteristic wheel-like appearance, which is diagnostic when visualized by electron microscope (EM). The icosahedral capsid is double-layered. The complete particle, designated as smooth, is 70 nm in diameter, but loss of the outer capsid reduces this to 55 nm, and the particle is then designated as rough. The spectrum of disease exhibited by infected individuals ranges from asymptomatic infection to fatal infection due to severe dehydration. The majority of symptomatic infections occur in children under five, in whom the disease is regarded as endemic. Incubation period is short (usually 12–24 hours) and the onset of diarrhoea is sudden. This typically lasts for four to seven days, but may

be longer if there is a history of malnutrition. Rotaviruses are particularly effective in producing dehydration (Wyatt *et al.*, 1979).

Serotyping divides rotaviruses into seven groups (A–G) and is based on the cross reactivity of the structural protein VP6, found in the inner capsid. Group A accounts for the vast majority of infection worldwide. However, group B has been associated with some large waterborne outbreaks in China (Hung, 1988). Group C has been responsible for a daycare outbreak in Brazil (Gabbay *et al.*, 1999). Group A rotaviruses can be further subdivided by typing based on the structural proteins VP4 and VP7, both of which are found on the outer capsid. VP4-specific types are designated as P (protease-sensitive protein or phosphoprotein) types, and VP7-specific types are designated as G (glycoprotein) types.

Rotavirus displays a marked seasonality in temperate climates, with the number of cases peaking in the colder winter months. In tropical climates, this seasonality is not as apparent, and infection may occur year round. Symptoms of rotavirus infection are non-specific and include vomiting and diarrhoea, occasionally accompanied by a low grade fever. Dehydration is more common with rotavirus infection than with most bacterial pathogens, and is the most common cause of death related to rotavirus infection. Treatment is non-specific and includes the use of oral rehydration therapy, especially in developing countries where malnutrition is common. (Maldonado and Yolken, 1990).

A study documenting rotavirus(RV) diarrhoea in Asian infants in South Africa, described the virological and epidemiological aspects of this disease in this population. Fifty five per cent of 1142 hospitalized cases investigated over a 31-month period showed positive stools for RV using ELISA. Most of these children stopped shedding RV by days 4-6 of hospital admission, though prolonged excretion was recorded in some

acute cases for up to 13 days. Mixed RV- bacterial infection occurred in 7% of the total gastro-enteritis(GE) patients, while 8.6% had pure bacterial gastro-enteritis. The age-groups mainly affected were between 3 and 14 months, with a peak at 9-11 months ; 3% of the rotavirus gastroenteritis (RVGE) patients were neonates. both the RVGE and the total gastroenteritis (GE) admission showed well-marked winter peak , with an inverse relationship between RV prevalence and both temperature and humidity. It was concluded that RV was the most important cause of infantile GE in this population, whereas pure bacterial infections played a relatively minor role (Haffejee and Moosa , 1990) .

In developing countries, however, a similar proportion will develop rotavirus diarrhoea, but one in eight will develop severe illness and one in 160 will die (Glass *et al.*, 1993) .Transmission occurs by the faecal–oral route and is thus more common in situations where sanitation is poor and the water supply is contaminated. Some speculate that a respiratory route is possible but this is not widely accepted.

Diarrhoea is mainly the result of a loss of extra cellular fluid, due to impaired absorption. Initially, the virus infects the tips of the villi in the proximal small intestine, resulting in shortening and a reduction in absorption capacity. The damaged cells are replaced by ones that are resistant to the virus and so, in the absence of severe dehydration, diarrhoea becomes self-limiting (White and Fenner, 1994).

Sixty-six stool specimens from infants with diarrhoea in Nigeria were examined for the presence of viral pathogens (Avery *et al.*, 1992). Rotavirus was found in 25.8% of specimens and astrovirus in 1.5%. Serotypes were determined for 47.1% of the rotavirus positive specimens, all of which were serotype 1. RNA analysis revealed no unusual electrophoretic profile. No enteric adenoviruses were detected. In contrast, in a parallel study conducted in the UK, rotaviruses (including

serotypes 1, 2 and 4) accounted for 21.9% of infections, adenovirus serotypes 40 and 41 for 13.6%, and astroviruses for 4.5% .

A survey of rotavirus infection in infants and young children with acute diarrhoea was made in Zaria, north Nigeria during 1979 and 1988. Total of 375 faecal specimens were collected from children aged between 1 and 60 months and 122 specimens from age-matched control children without diarrhoea and 14 specimens were collected from neonates in the University Teaching Hospital. Rotavirus antigen was detected in 61 diarrhoeal and four control specimens; four neonates were shedding rotavirus. Polyacrylamide gel electrophoresis of the viral genome showed the presence of five strains of rotavirus with long RNA electropherotypes and one short pattern (Pennap *et al.*, 2000) .

Sixty-five episodes of rotavirus diarrhoea in Nigeria , detected during a longitudinal follow-up of 336 infants from birth to 24-32 months of age , were analyzed for clinical symptoms. Rotavirus gastroenteritis was characterized by watery diarrhoea , vomiting (particularly in older children) , fever and dehydration (Ruuuska and Vesikari, 1990).

A study was made by Kohler *et al.*, (1990) of small intestinal mucosal biopsies in 40 children with acute rotavirus diarrhoea. Biopsies were taken from these children between the 2nd to 10th day of acute phase. Their age was less than 18 month. Among these childrens 95% of the patients had normal histological findings of the small intestinal mucosa. Only 2 children (5%) had well defined injuries of intestinal mucosa. In one child with gastroenteritis rotaviruses were found in cerebrospinal fluid. In 31.25% of all children rotavirus infection was acquired by a nosocomial infection.

From March to september 1988 stool specimens of 101 hospitalized diarrhoeic infants and children, aged 1-24 months were

examined by enzyme-linked immunosorbent assay(ELISA) for detection of the presence of rotavirus antigen. This agent was found in 40(40%) of the 101 episodes of acute diarrhoea, and strains were both characterized by analysis of RNA in polyacrilamide gel and serotyped by ELISA using serotype-spific monoclonal antibodies. The highest frequency of rotavirus positivity was 80 % in the 16-18 month age group. All 11(28 %) serotyped strains belonged to serotype 3 whereas absence of Vp7, the major outer capsid glycoprotein, did not allow serotyping in 29(73 %) of the 40-positive specimens (Linhares *et al.*, 1993) .

Four hundred and forty-two samples from children , age from birth to 5 years old , with acute diarrhoea attending hospitals and clinics in Jos in Nigeria between May 1986 to April 1987 were examined for the presence of rotavirus by the enzyme linked immunosorbent assay . One hundred and forty-six of these samples were positive; giving a prevalence rate of 33 %. The virus was more prevalent in infants 1-6 months old and decreased with an increase in age . Rotavirus was found to occur throughout the year , but there was a much higher prevalence of the virus during the dry season (59 %) than in the rainy season (21 %) relative humidity being the most influential climatic factor for this variation . Male children and breast-fed children were more predisposed to infection with rotavirus than their counterparts (Gomwalk.,*et al* 1990) .

Rotaviruses were studied in a cohort of children from Gaza, during their fist year of life (Simhon *et al.*, 1990). Surveillance was effected through visits to the local health clinic by parents and infants, and to a lesser extent , field workers'home visits . The observed rate of diarrhoea (all causes) , and of rotavirus-associated diarrhoea was 1.25 and 0.1 episode per child-year, respectively . Of the 130 diarrhoea episodes in the cohort, only 6.9% were rotavirus-associated . Only nine (37.5%) of 24 children in whom rotavirus antigen was detected experienced- about of

diarrhoeal illness. However, 59.2% of the group of children had rotavirus serum antibodies by one year of age. The data indicate that rotavirus excretion in Gaza children tends to be asymptomatic during the first year of life.

A Latex agglutination (LA) test , using latex beads sensitized with anti-rotavirus immunoglobulin G, was evaluated to detect human rotavirus in 200 stool specimens by comparing its results with those of an ELISA (Rotazyme, Abbott Laboratory, Diagnostic Div., North Chicago, IL). The specimens were collected from a 200 infants and pre-school children attending the Diarrhoeal Disease Research and Rehydration Center at the Bab-EL- Sha'reya University Hospital, Egypt (Amer *et al* .,1990) . Of 2000 stool specimens were tested, 79 were positive by the ELISA and 68 were positive by the LA test . Taking the ELISA as the standard, the LA test showed 11 false-negative and six false-positive giving a sensitivity and specificity of 86% and 95% respectively. Using 48 stool specimens positive for rotavirus by both the tests, the degree of positivity of the LA test roughly showed a linear relationship with the degree of rotazyme optical density. Thus, the simple and inexpensive LA test may be useful as screening procedure to detect rotaviruses in the stools of children with diarrhoea.

In 1998, a tetravalent human/rhesus monkey rotavirus (HRRV)-based vaccine received US Food and Drug Administration (FDA) approval. However, following administration to approximately one million infants at two, four and six months, the Advisory Committee on Immunisation Practices (ACIP) concluded that the frequency of intussusception was significantly increased following vaccination and withdrew recommendation for its use. Other candidate vaccines, such as a live oral vaccine using a lamb rotavirus, have been investigated. (Bresee *et al* .,1999). It is thought that a successful vaccine stimulates IgA

production and other forms of local immunity, as studies with animal models indicate that local intestinal immunity plays the prime role in resistance.

2.2.2 Adenoviruses:

These are DNA viruses of the adenoviridae family and have 49 serotypes, which are divided into six groups (A–F). The viral particles are icosahedral and have a diameter of 70–80 nm. Adenoviruses are widely recognized causes of respiratory, ocular, and genitourinary infections. However, serotypes 40 and 41 (previously called fastidious enteric adenoviruses) primarily affect the gut, contributing to 5%-20% of hospitalizations for childhood diarrhoea in developed countries (Uhnou *et al.* , 1984). Peak incidence is among children less than 2 years of age, but older children and adults may be infected, with or without symptoms. Infections occur throughout the year with no clear peaks. Other serotypes of adenovirus, particularly 31, have also been associated with diarrhoea (Hammond *et al.* ,1987) .

2.2.3 Caliciviruses:

The caliciviruses are RNA viruses that cannot be cultivated in cell culture. A British study suggested that approximately 3% of children hospitalized for diarrhoea excrete calicivirus (Ellis *et al.* , 1984). and a U.S. study found approximately the same percentage (2.9%) for children with diarrhoea in day-care centers (Matson *et al.*, 1989). On the basis of antibody-prevalence studies of pooled immunoglobulin and serum samples from many parts of the world, most persons appear to have been infected by age 12, and the peak acquisition takes place between 3 months and 6 years (Cubitt *et al.* , 1987). Seasonality is unknown.

2.2.4 Norwalk-like virus:

The Norwalk virus is the representative agent of a heterogenous

group of viruses, also called small round structured viruses (SRSVs) or the Norwalk-like family of agents. The antigenic interrelationships among the many members of this class are complex, and the agents are usually identified by where outbreaks of diarrhoea occur. Although Norwalk-like viruses may play a role in endemic diarrhoea, diagnostic technology is not yet sufficiently developed to assess the aspect of their disease burden. In the United States, illness is most commonly reported among persons of school age and older. Levels of antibody specific to the Norwalk agent are low during childhood but reach 50% by middle age (Blacklow *et al.*, 1979) . In developing countries, antibodies are acquired at an earlier age; peak incidence of illness may also occur among younger age groups than in developed nations (Greenberg *et al.*, 1979).

2.3 Other viruses:

Several other agents, listed below, have been implicated in viral gastroenteritis, but their public health importance is not yet clear.

2.3.1 Pestivirus:

The genome of a cytopathogenic Pestivirus isolate , is characterized by cells contained viral genomic RNA of 12.3 kb and also Pestivirus contained viral genomRNA of 8 kb. (N Tautz *et al* ., 1994)

A recent study showed that in an Arizona indian reservation, 23% of specimens from children less than 2 years of age with gastroenteritis of unknown etiology were antigen-positive for pestivirus, compared with 3% of controls. Illness was relatively mild, and duration was 3 days . (Yolken *et al* .,1989). Antibody studies of serum samples from Arizona, Maryland, and Peru suggested that 30-50% of children and adults had been infected, with peak exposure occurring at less than 2 years of age

(Yolken *et al.*, 1988.).

2.3.2 Picobirnavirus

Picobirnavirus (PBV) was first described by Pereira *et al.*, (1988) who detected two bands of double-stranded RNA genome by polyacrylamide gel electrophoresis (PAGE) in fecal samples from children . Reports from Brazil documented human cases of diarrhoea caused by picobirnavirus, which had been thought to be a cause of diarrhoea only in animals. The importance of this pathogens is unknown.

2.3.3 Parvovirus

Parvovirus B19 (B19V), the only member of the Parvoviridae family known to cause disease in humans, is a single-stranded DNA virus. Parvovirus-like particles have been identified by electron microscopy in stool specimens of both well and ill persons in Britain (Flewett *et al.*, 1974). The relationship of these particles to disease is unclear, but they have been associated with shellfish-related outbreaks of gastroenteritis (Appleton, 1987)

2.3.4 Enteroviruses

Enteroviruses are icosahedral nonenveloped viruses that are approximately 30 nm in diameter. The genome is made of a single-stranded linear molecule of RNA. Enteroviruses resist lipid solvents and tolerate a wide range of pH and temperature . Enteroviruses cause a wide spectrum of disease, in which gastroenteritis plays a minor role . Although the entry of polio, coxsackie, echo, or other enteroviruses through the gut may cause incidental mild diarrhoeal symptoms, the spread of the virus through the bloodstream to other organs (e.g., central nervous system, heart, pleura, pancreatic islets) produces major disease manifestations. Although reports have linked some enteroviruses to

illnesses in which diarrhoea was the sole symptom, an outbreak or case of gastroenteritis should not be attributed to an enterovirus merely because it was isolated in the stool of an affected person (Birch *et al.*,1977).

2.3.5 Torovirus

Toroviruses are spherical, oval, elongated, or kidney-shaped enveloped viruses with a single-stranded RNA genome of positive polarity; they belong to the second genus in the family Coronaviridae. Toroviruses are known causes of diarrhoea among cattle, and identification in human specimens has been reported (Beards *et al.*,1984). Antibodies to the Breda strain of toroviruses were not present. The torovirus importance as a cause of human disease is unknown (Brown *et al.*,1987).

2.3.6 Coronavirus

Coronavirus is a genus of animal virus belonging to the family Coronaviridae. Coronaviruses are enveloped viruses with a positive-sense single-stranded RNA genome and a helical symmetry. Coronaviruses are well-established causes of diarrhoea in animals and respiratory disease in humans. These viruses have been identified in the stool of persons with gastroenteritis (usually children less than 2 years of age), but human controls have been found to shed them with higher frequency (Kidd *et al.*,1989), raising doubt about their etiologic role in human diarrhoea. Coronaviruses have been detected most frequently in the southwest in U.S.A; one group reported that more than two-thirds of diarrhoeal stools examined by electron microscopy over an 8-year period contained such viruses, although no comparison was made with specimens from well persons (Payne *et al.*,1986).

2.4 Association between viruses and bacteria in causing

diarrhoea:

Adiarrhoeal disease survey in Alexandria, Egypt determine the prevalence, seasonality and household risk factors for *campylobacter Spp* and other microorganisms associated with diarrhea in children. The study population was 880 children. The results showed an overall isolation frequencies for *campylobacter spp* of 16.8% for cases. Other enteropathogens detected in the same diarrhoeal stools were: rotavirus (28.6%), *Giardia lamblia* trophozoites(21.3%), enterotoxigenic *E. coli* (8.7%), *Entamoeba histolytica* (3.9%), *Salmonella spp.*(2.7%), and *Shigella spp.*(1.8%). There were few or no isolates of *Aeromonas spp.*, *Vibrio spp.*, *Yersinia Spp.*, or *Plesiomonas spp.* Comparisons among cases showed that *Campylobacter spp* . isolations were more prevalent during the rainy season and positively associated with keeping fowl in the home or having an outdoor source of drinking water. Patients with campylobacter-positive diarrhoeal stools were frequently co-infected with rotavirus (28.6%) or *G.lamblia* (24.5%) (Pazzaglia *et al.*, 1993)

To determine the incidence, clinical significance and etiology of diarrhoea in early childhood, a cohort of 336 children were followed from birth to the age of 24-32 months. More than half (55%) of the children had no diarrhoea, 26% had one episodes and 195 had two or more episodes of diarrhoea during follow-up; altogether 248 episodes of diarrhoea were detected . Rotavirus was by far the most common (26%) identified pathogen; adenovirus were detected in 4% and bacterial pathogens enteropathogenic *E. coli* (EPEC), salmonellae and yersiniae in 4% of the cases. Two thirds of the episodes remained etiologically unresolved. Rotavirus diarrhoea was significantly more severe than diarrhoea due to other causes ; 75% of severe episodes of diarrhoea were associated with rotavirus. About two thirds of the infants were breast-fed

over 6 months; breast-feeding for less than 6 months was associated with higher incidence of rotavirus diarrhoea between 7-12 months of age but not thereafter . About three quarters of the children were cared for beyond 12 months of age ; those at home had lower rate of rotavirus diarrhoea than those at day-care centers (Ruuska and Vesikari, 1991).

A recognized pathogen was detected in 78% of the diarrhoeic patients , 6% of whom had a mixed infection with two agents. Enteropathogenic *E. coli* (EPEC) was the most common enteropathogen detected (22%), followed by rotavirus (19%), and adenovirus(10%). Altogether 6% had diarrhoea associated with salmonella or shigella and 3% showed diarrhoeal illness associated with astrovirus. Infants less than 6 months of age were most commonly infected with enterobacteria (35%), mainly enteropathogenic *E. coli*, whereas children 6 months to 2 years presented more often with viruses, mainly rotaviruses. Enteropathogens were found during all seasons of year and rotaviruses showed a seasonal variation (Huilan *et al* , 1991).

2.5 Diarrhoea in infants in sudan:

Erwa (1969) made a clinical and bacteriological examination on 150 children suffering from diarrhoea and on 50 children not suffering from diarrhoea. All children were of the age group from birth to 3 years .Rectal swabs taken and examined bacteriologically, revealed the presence of pathogen in 37% of the first group with enteropathogenic *E. coli* as the predominantly prevalent organism . The rate of isolation of pathogen in the second group (50 children) was 12% and again *E. coli* gave the highest proportions. Antibiotic sensitivity test showed a high degree of resistance by *E. coli* and shigella to the majority of antibiotics included in the test. Multiple resistance was also evident. The clinical presentation of the disease showed no variation with the bacterial

aetiological agents.

The same author in December 1975 investigated 654 children suffering from diarrhoeal diseases. He found that bacterial agents were the most important aetiological factors in over 72% of the patients examined. The commonest pathogens were enteropathogenic *E. coli* strains (27.8%) followed by *Shigella* strains (8.2%) and *Salmonella* strains (2%). Mixed infection with these organisms also occurred. Potential pathogens such as enterococci, *Proteus spp*, and *Pseudomonas aeruginosa* were also isolated. Aerogenic *Sh. flexneri* and certain salmonella species were also isolated. Enterobacteria in diarrhoea were serologically typed, and there was a high degree of antibiotic resistance by *E. coli* serotypes but less so in the case of shigella.

Diarrhoeal diseases are noted to be a major cause of morbidity in children below five years in Juba. *Shigella spp* were found to top list after the 1st six months of life and enteropathogenic *E. coli* were more prevalent among children below the one year of life, infants-salmonellosis was the least cause of bacterial diarrhoea in children below the age of 5 years in Juba. (Sharaf, 1984).

Giardiasis was the major intestinal protozoa in all ages between 6 months and five years. Amoebiasis is common in Juba children. And malaria as a single cause of diarrhoea was reported (Sharaf, 1984).

The epidemiology of gastroenteritis (diarrhoea) in children less than 5 years of age in Sudan was studied during 2002-2004 by Ali *et al.*, (2004) (personal communication). They found that of a total of 134 stool samples from diarrhoeic children in different localities in Sudan (Khartoum, River Nile and White Nile States) tested for group A rotavirus using ELISA, 35 gave positive results. Twenty nine of these were examined by PAGE. Twenty four of them were positive with long electropherotype and five had different profiles. The positive samples are

showed the characteristic appearance of rotavirus under electron microscope.

Chapter Theree

Materials and Methods

3.1- Bacteriology:

3-1.1 Materials :

3.1.1.1 Reagents:-

- | | |
|------------------------------------|---|
| 1- Hydrogen peroxide | The British Drug House chemical (BDH) U.K. |
| 2- Sulphuric acid | The British Drug House chemical (BDH) U.K |
| 3- Immersion oil | The British Drug House chemical (BDH) U.K |
| 4- Crystal violet | Hopkins and Williams Ltd U.K. |
| 5- Gram's Iodine | Hopkins and Williams Ltd U.K. |
| 6- Safranine red | BDH Chemicals Ltd. Poole England |
| 7- Alcohol (Methanol and Ethanol) | Hopkins and Williams Ltd U.K. |
| 8- Acetone | Hopkins and Williams Ltd U.K. |

9- Kovac's reagent :

To make 10 ml :

4- Dimethylaminobenzaldehyde 2g.

(para- dimethylaminobenzaldehyde).

Isoamyl alcohol (3- Methyl- 1- butanol) 30ml.

Hydrochloric acid , concentrated 10ml (Cheesbrough, 2000)

10- Oxidase reagent :

To make 10 ml:

Tetramethyl- p- phenylenediamine dihydrochloride..... 0.1g

Distilled water.....10ml.

(Cheesbrough, 2000)

11- Urea powder:

To make 1 lit of MIU base medium:

Tryptone 30mg.
Potassium dihydrogen phosphate 1g.
Sodium chloride 5mg.
Agar 4g.
Phenol red 2.5g/l (0.25%) w/v 2ml.
Distilled water 1lit. .
(Cheesbrough, 2000)

3.1.1.2 Culture media :

The following culture media were obtained from Oxoid Ltd U.K.
and they were prepared as instructed by the manufacturer:

3.1.1.2.1 Blood agar : code No : CM55

Forty grams of blood agar base were suspended in 1 lit of distilled water, boiled to dissolve the powder completely and then sterilized in the autoclave at 121°C for 15 minutes. The medium was then cooled to 45° - 50°C before addition of 7% defibrinated blood, mixed gently and dispensed into sterile Petri dishes.

3.1.1.2.2 MacConkey's agar code NO CM7b

Forty-seven grams of the powder were suspended in 1 lit of distilled water, boiled to dissolve the, then sterilized in the autoclave and mixed well and poured. The surface of the medium was left to dry before inoculation.

3.1.1.2.3 Muller- Hinton agar: code No : DM170

Thirty-five grams of the powder were suspended in 1lit of distilled water, boiled to dissolve the powder completely, then sterilized in the autoclave. The inclusion of starch ensures that toxic factor found during growth will be absorbed and it's presence is often essential to establish growth from very small inocula. Muller-Hinton agar was used for

antibiotic sensitivity testing.

2.1.1.2.4 Nutrient agar: code No : CM3

Twenty- eight grams of the powder were suspended in 1 lit of distilled water, boiled to dissolve the powder completely, then sterilized in the autoclave . The medium was dispensed aseptically in the required amounts i-e 3 ml to make nutrient agar slopes and 5 ml to make nutrient agar deeps and then autoclaved (Cheesbrough, 2000)

3.1.1.2.5 Selenite F broth:

Selenite F (faeces) broth was used as an enrichment medium for the isolation of salmonella species from faecal specimens .

Sodium hydrogen selenite (0.8 g) was added to 1 gram peptone and 0.8 grams of mannitol and then 2 grams of di-sodium hydrogen phosphate were added . The mixture , were suspended in 200 ml distilled water , boiled to 80c to dissolve and dispensed in 7 ml values in test tubes before autoclaving (Cheesbrough, 2000) .

3.1.1.2.6 Semi-solid medium:

Thirteen grams of nutrient broth (**code No : CM76**) with 7.5 grams of agar were suspended in 1 lit of distilled water, boiled to dissolve and then dispensed into sterile tubes and then autoclaved.

3.1.1.2.7 Koser's Citrate Medium: code No : CM155

It is a fluid medium used in the citrate utilization test. One lit of distilled water was used to suspend 5.2 grams of the powder, boiled to dissolve the medium completely. The medium was distributed in 3ml amount in a small screw-cap bottles and then sterilized in the autoclave .

The medium was inoculated by straight wire, so as to avoid the carrying of nutrients from the culture, also a light inoculum was used and the bottle caps were left loose during incubation period.

3.1.1.2.8 Peptone water: code No : M104

Peptone water was used to test indole production (Cowan and Steel, 1975). Fifteen grams of the powder were suspended in 1 lit of distilled water, boiled to dissolve the medium completely, The medium was distributed in tubes and then sterilized in the autoclave at 121C for 15 minutes.

3.1.1.2.9 SS agar:- code No : CM99

Salmonella-Shigella agar is a selective medium used to isolate salmonella and shigella species from faecal specimens (Gillespie, 1994). Fifty-seven grams of the powder were suspended in 1 lit of distilled water, heated with great care so that not to boil. As soon as the medium has cooled to 50– 55C, it was mixed well, and dispensed aseptically in sterile Petri dishes.

3.1.1.2.10 Kligler Iron Agar (K.I.A) code No : CM33

Kligler Iron Agar is a differential slope medium used to assist in the identification of salmonella, shigella, and other enteric bacteria. K.I.A reactions are based on the fermentation of lactose and glucose (dextrose) with the production of hydrogen sulphide :

Five grams of the powder were suspended in 100 ml of distilled water, boiled to dissolve the medium, dispensed in tubes and then sterilized by autoclaving at 121C for 15 minutes and then allowed to set in the slanting position.

3.1.1.2.11 Urea agar: code No : CM53

Twenty-four grams of the powder were suspended in 950 ml of distilled water, boiled to dissolve the medium completely, and then sterilized in the autoclave at 115°C for 20 minutes. Then it was cooled to 50°C and aseptically 50 ml of sterile 40% urea solution were added (FDO48), mixed well, and then dispensed into sterile tubes and allowed to set in the slanting position.

3.1.2 Methods:

3.1.2.1. Sterilization:

- 1- Glassware, Petridishes, pipettes, flasks, test tubes, bottles ...etc were sterilized in the hot air oven at 160C for one hour.
- 2- The media, solutions, screw-capped bottles, rubber- stoppered flasks- etc were sterilized in the autoclave at 121°C for 15 minutes.
- 3- Straight wires and platinum loops were sterilized by direct Bunsen burner flame.
- 4- Disinfecting: phenolic disinfectant 5% for clean situations was used for disinfecting floor, walls and the roof of the laboratory, also alcohol 70% was used for disinfecting the benches.
- 5- Hands and utensils were washed by medicated soaps, and dried by goose, cotton and even air.

3.1.3. Staining Techniques

3.1.3.1 Preparation of smears:

Bacterial smears were prepared by emulsifying small inoculum from the bacterial culture in a drop of normal saline and spread evenly on a clean slide. The smears were allowed to dry in air and then fixed by gentle heating (passing the slide quick enough three times through the flame)

3.1.3.2 Gram's Stain:

Gram's stain method in the was done according to the method described by Cruickshank *et al.*, (1982) as follows:

Smears were prepared in clean slides, allowed to air dry, fixed by heating and stained by the gram method, the smears were placed on the rack horizontally, then flooded with crystal violet stain (basic stain) for one minute, and then the stain was washed by water. The smears were covered with Gram's iodine for one minute, rinsed with water. Alcohol (70% ethanol) or acetone was

applied for decolourization for up to 15 seconds and then the slides were rinsed with water again. Safranine (counter stain) was added and left for 60 seconds, then rinsed with water and allowed to dry. Smears were examined by the microscope under the oil immersion lens. Bacteria coloured red were labeled as Gram-negative and violet coloured as Gram-positive.

3.1.4 Collection and Culture of Specimens:

Faecal specimens were collected from 60 children 4 months to 5 years of age suffering from diarrhoea and were admitted to hospitals. Samples were collected in sterile containers and transported directly to the laboratory.

Samples were collected from both sexes during the period from May to June 2006 from Ibraheem Malik hospital (Khartoum), Khartoum Teaching Hospital and Ahmed Qasim Hospital for children (Khartoum North).

All samples were cultured on plates containing the appropriate differential media (SS agar, MacConkey's agar, Nutrient Agar and Blood Agar) and Selenite F broth. After streaking and culturing the samples to obtain single colonies of suspected organisms , the plates were incubated at 37C for 24 hours under aerobic conditions .

3.1.5 Examination and Preservation of Cultures:

After the end of the incubation period, the plates were examined with naked eye for significant growth and for any changes in the media. Pure colonies were subcultured on nutrient agar slopes and incubated at 37°C for 18-24 hours and then preserved at 4°C in the refrigerator for further investigations.

3.1.6 Identification of Clinical Isolates:

3.1.6.1 Primary Identification

Primary identification was done by examination with naked eye for colonial, lactose-fermenting and motility. The smell of the culture was also tested. This was followed by microscopic examination of gram stained smears.

3.1.6.2 Secondary Identification

Secondary identification was done using biochemical tests:

3.1.6.2.1 Catalase Test:

The test was done to differentiate those bacteria that produce the enzyme catalase from the non-catalase producing bacteria. The test was done by taking a small portion of colonies of the test organism with a sterile wooden stick which was immersed in a tube containing 3% hydrogen peroxide solution. Immediate bubbling of O₂ indicated a positive catalase test (Cheesbrough,2000).

3.1.6.2.2 Oxidase Test:

This test detects the presence of the enzyme oxidase. The enzyme catalyses the transport of electrons between electron donors in the bacteria . In the test, redox dye = tetramethyl-p- phenylene-diamine is reduced to a deep purple colour (Collee *et al.*, 1996).The test was done by the wet filter paper method.

A strip of filter paper was soaked in 1% solution of the reagent and then at once used by rubbing a speck of culture on it with a platinum loop.

A positive reaction was indicated by an intense deep-purple hue, appearing within 5-10 seconds, a delayed positive reaction by colouration in 10- 60 seconds, and a negative reaction by absence of colouration or by colouration later than 60 seconds, (Collee *et al.*, 1996)

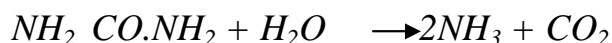
3.1.6.2.3: Citrate Utilization:

This test is used to differentiate enterobacteria that utilize citrate from other bacteria that do not utilize citrate (Cowan and Steel, 1975).

Koser's citrate medium was inoculated and examined daily up to 7 days for turbidity. (Cowan and Steel, 1975)

3.1.6.2.4: Urease Test :

Bacteria, particularly those growing naturally in an environment exposed to urine, may decompose urea by means of the enzyme urease, (Mackie and McCartney, 1996)



The tubes were inoculated heavily over the entire slope surface and incubated at 37°C. Urease positive organism changed the color of the medium to purple-pink (Collee *et al.*, 1996).

3.1.6.2.5. Indole Test :

The indole test is important in the identification of Enterobacteriaceae. It is used to differentiate Gram-negative rods (Gillespie, 1994). Peptone water in a test tube was inoculated by the culture, and then incubated for 24 hours at 35°C. Kovac's reagent was added in a few drops. In the positive tubes a red ring immediately appeared while no color was observed in the negative tubes (Gillespie, 1994).

3.1.6.2.6 Motility Test:

Semi-solid medium were inoculated by the test organisms using a straight wire and then incubated for 24 hours at 37°C. The spreading of the growth in the semi-solid agar was regarded as positive (Cowan and Steel, 1975).

3.1.6.2.7 Kligler Iron Agar (K.I.A) Test :

This is suitable to detect hydrogen sulphide production, fermentation of lactose and glucose and production of gas (Cheesbrough, 2000)

With a straight wire, KIA medium was inoculated first by stabbing the butt and then streaking the slope in a zig-zag pattern. The results were read as follows:

- .Yellow butt and red slope indicated fermentation of glucose only.
- .Yellow butt and yellow slope indicated fermentation of lactose and possibly glucose.
- .Red butt and red slope indicated no fermentation.
- .Blackening of the medium indicated hydrogen sulphide (H₂S) production
- .Cracks and bubbles indicated gas production (Cheesbrough, 2000)

Antibiotic Susceptibility pattern:

Antitiotic susceptibility of Enterohacteriaceae was tested using Kirb y-Bauer disc diffusion method.

3.1.7 Disc diffusion susceptibility test:

3.1.7.1 Preparation of inoculum:-

Selected colonies were subcultured on nutrient agar and incubated at 37C for 24 hours. and the growth was transferred to test tubes containing 4 ml of sterile normal saline. The tubes were incubated for 24 hours at 37C to allow the microorganisms grow well.

3.1.7.2 Antibiotic discs:

Commercially prepared discs dispensers were used form (Oxoid) which produces discs with accurate antibiotic contents coded as recommended by W.H.O Discs and disc dispensers were stored at a temperature less than 8 C. The antibiotics contained in the disc were :

- | | |
|----------------------------|---------|
| 1.Ampicillin/Sulbactam(AS) | 20 mcg. |
| 2.CO-Trimoxazole(BA) | 25 mcg. |
| 3.Cefotaxime(CF) | 30 mcg. |

4.Pipracillin/Tazobactam(TZP)	100/10 mcg.
5.Chloramphenicol(CH)	30 mcg.
6.Ciprofloxacin(CP)	5 mcg.
7.Ceftizoxime(CI)	30 mcg.
8.Tetracycline(TE)	30 mcg.
9.Ofloxacin(OF)	5 mcg.
10.Gentamicin(GM)	10 mcg.
11.Amikacin(AK)	30 mcg.
12.Pefloxacin(PF)	10 mcg.

Before use, the disk containers were allowed to warm up slowly at room temperature to minimize condensation of moisture, which may lead to hydrolysis of antibiotic (Mackie and McCartney, 1996).

3.1.7.3 The procedure:

Sterile cotton wool swabs dipped into the inoculum suspension and then rotated several times with firm pressure on the inside wall of the tube to remove the excess fluid. The dried surface of muller Hinton agar plates were inoculated by streaking the swabs over the half entire agar surface, then rubbed to the whole surface of the plate. The plates were rotated 60° to ensure an even distribution of the inoculum. Then the lid of the plates were replaced partially and allowed for 30 minutes to dry at room temperature.

Aseptically the discs were placed on the inoculated surface of agar about 15 mm from the edges of the plate by using sterile forceps and disc dispenser. The plates were inverted within 30 minutes of applying the discs and incubated aerobically at 37C for 18-24 hours.

3.1.7.4 Interpretation (reading the plates):

After overnight incubation , the diameter of each zone of inhibition was measured three times with a ruler on the underside of the plate from the edge of the disc to the edge of inhibition zone .

The results were recorded as:

- Susceptible (S): Zone radius wider than, equal to, 3 mm .

-Intermediate (I): Zone radius equal 3mm, but not less than 3 mm.

Resistant (R): No zone of inhibition or zone radius measures 2 mm or less.

Referring to interpretive chart of the National committee for clinical laboratory standards (NCCLS) . (Cheesbrough, 2000).

3.2 B/Virology:

For the diagnosis of rotavirus , double antibody (sandwich) ELISA was used for the qualitative determination of rotavirus antigen in feces. A polyclonal anti-rotavirus antibody was used to capture the antigen from the stool supernatant (Kapikian *et al.*, 1980) .

3.2.1 Principle of Procedure:

Rotavirus antigens present in the stool supernatant are captured by antibodies attached to the wells and then polyclonal anti-rotavirus (antibody) that sandwiches the antigen was added with Horseradish peroxidase simultaneously (conjugate) .

After washings to remove unbound enzyme, a chromogen is added which develops a blue color in the presence of the enzyme complex and peroxide. The stop solution ends the reaction and turns the blue color to yellow.

3.2.2 Materials:

Commercial Kit provided by DAKO ltd (United Kingdom)

3.2.2.1 Reagents:

- **Test strips:** microwells containing anti-rotavirus polyclonal antibodies - 96 test wells.

-**Reagent 1:** 110 ml sample diluent: tris buffered saline containing antimicrobial agent and a red dye.

- **Reagent 2:** 5 ml positive control : inactivated bovine rotavirus (calf rotavirus strain 3209176) in buffer containing antimicrobial agent and red dye. Prior to inactivation , positive material was shown to contain approximately 10^5 infectious forming units per ml.

-**Reagent 3 :** 13 ml conjugate : rotavirus specific rabbit polyclonal antibody conjugated to horseradish peroxidase in buffered protein solution containing antimicrobial agent and blue dye.

-**Reagent 4 :** 50 ml washing buffer concentrate (x25): tris buffered solution containing antimicrobial agent and detergent.

-**Reagent 5:** 13 ml substrate : stabilized peroxidase and 3.3-5.5 % tetramethylbensidine (TMB) in dilute buffer solution . TMB has been reported to be non-carcinogenic. However, personal protective equipment is recommended to avoid direct exposure.

-**Reagent 6:** Stopping solution : 12 ml stopping solution : 0.46 mol/l sulphuric acid.

3.2.3 Equipments:

-Pipettes

-Squeeze bottle for washing strips

-spectrophotometer

-Graduated cylinder

3.2.4 Methods:

3.2.4.1 Storage Conditions

Reagents, strips and bottled components were Stored between 2 - 8° C in a refrigerator. Squeeze bottle containing diluted wash buffer are stored at room temperature.

3.2.4.2 Wash/Dilution Buffer:

Provided x25 concentrated . Dilute washing buffer was concentrated by adding 1 part concentrate to 24 parts fresh distilled water that to provide 100 ml of working strength washing buffer for 5 washes of each –well strip.

3.2.4.3 Collection of Stool :

Stools were collected in clean containers. Samples were frozen. at -20C until used.

3.2.4.3.1 Preparation of Sample:

Frozen stools were thawed at room temperature, and 1:5 dilution of stool was made by adding 1 gram to 4ml of diluted wash buffer. This was mixed well and the heavy particulates were allowed to settle.

3.2.4.4 Procedure:

- 1- Hundred µl (2 drops) of the negative control was added to well 1 and 100 µl (2drops) of the positive control added to well 2 (both used as undiluted).
- 2- Hundred µl (2drops) of the stool supernatant was added to the test wells.
- 3- Hundred µl (2drops) of conjugate was added to test wells.
- 4- The wells were Incubated at room temperature for 60 minutes at 30C.
- 5- The wells were washed 5 times using working strength washing buffer .
- 6- Two drops (100µl) of substrate was added to each wells.
- 7- The wells were incubated at 30C for 10 minutes.
- 8- The result read visually immediately.

9- Two drops of stopping solution then added .

10- Result read photometrically at 450nm.

3.2.5 Interpretation of Results – Visual:

3.2.5.1 Reactive color:

Any sample well that had distinct and substantial yellow color was considered positive.

3.2.5.2 Non reactive color:

Any sample that did not have a distinct yellow color was considered negative.

3.2.5.3 Interpretation of Results - ELISA Reader:

all wells were read using a bichromatic reading with filters at 450nm .

-Absorbance reading of 0.15 and above indicated that the sample contained rotavirus antigen.

- Absorbance reading less than 0.15 indicated that the sample did not contain detectable levels of rotavirus antigen.

Chapter four

Results

4.1 Bacteriology:

4.1.1 Identification of the clinical isolates:

It was found that out of the 60 clinical isolates for 60 faecal samples , 60 were Gram negative rod which included : *E. coli* associated with 5 *Salmonella typhi* isolates (8.3%) , 5 *Salmonella paratyphi* isolates (8.3%) , 4 *Salmonella typhimurium* isolates (6.7%), one *Shigella dysenteriae* isolate (1.7%) , 2 *Shigella flexneri* isolates (3.3%) , one *Shigella boydii* isolates (1.7%) , 28 *Escherichia coli* isolates (46.7%) as pure culture , 2 *proteus vulgaris* isolates (3.3%) , 4 *Klebsiella pneumoniae* isolates (6.7%) , 2 were *Klebsiella rhinoscleromatis* isolates (3.3%) and 6 isolates of *Pseudomonas aeruginosa* (10%). (Table 1 and 2):

Table 1 :

Growth of isolated bacteria in different culture media :

Serial No.	Organism	Media		SS . A	S. F.B	B. A
		N. A	Mac. A			
1	<i>Salmonella typhi</i> (5)	+	N. L	+	+	+
2	<i>S. paratyphi</i> (5)	+	N.L	+	+	+
3	<i>S. typhimurium</i> (4)	+	N. L	+	+	+
4	<i>Shigella dysenteriae</i> (1)	+	N.L	+	+	+
5	<i>Shi. Boydii</i> (1)	+	N.L	+	+	+
6	<i>Shi. Flexneri</i> (2)	+	N.L	+	+	+
7	<i>Proteus vulgaris</i> (2)	+	N.L	-	-	+
8	<i>Escherichia coli</i> (29)	+	L.F	-	-	+
9	<i>Klebsiella pneumonia</i> (4)	+	L.F	-	-	+
10	<i>K.rhinoscleromatis</i> (2)	+	L.F	-	-	+
11	<i>Pseudomonas aeruginosa</i> (6)	+	+	-	-	+

Key:

N.A : Nutrient Agar

Mac. A: MacConkey Agar

SS.A: Salmonella

Shigella Agar

S.F.B: Selenite F Broth

B.A : Blood Agar
 N.L: Non Lactose fermenting (white colonies)
 L.F: Lactose Fermenting (pink colonies)
 + : Growth
 - : No growth

Table 2:
biochemical Characteristics of the isolated bacteria in the tests:

		K.I.A				Fermentation of sugars									Urea
Serial No.	Organism	Slope	Butt	H2S	Gsa	Lac	Man	Glu	Suc	Ox	Cat	Cit	Mot	Ind	
1	<i>S. typhi</i>	R	Y	+	-	-	+	+	-	-	+	-	+	-	-
2	<i>S.paratyphi</i>	R	Y	-	+	-	+	+	-	-	+	-	+	-	-
3	<i>S.typhimurium</i>	R	Y	+	+	-	+	+	-	-	+	-	+	-	-
4	<i>Sh. dysenteriae</i>	R	Y	-	-	+	+	+	-	-	+	-	-	-	-
5	<i>Sh. boydii</i>	R	Y	-	+	+	+	+	-	-	+	-	-	+	-
6	<i>Sh. flexneri</i>	R	Y	-	+	+	+	+	-	-	+	-	-	-	-
7	<i>Proteus vulgaris</i>	R	Y	+	D	+	+	+	-	-	+	D	+	+	+
8	<i>E. coli</i>	Y	Y	-	+	+	+	+	-	-	+	-	+	+	-
9	<i>K. pneumonia</i>	Y	Y	-	+	+	+	+	+	-	+	+	-	-	+
10	<i>K.rhinoscleromatis</i>	Y	Y	-	-	+	+	+	+	-	+	+	-	-	+
11	<i>Ps. aeruginosa</i>	R	R	-	+	-	-	D	-	+	+	+	+	-	D

Key :

R: Red Y: Yellow K.I.A: Kligler Iron Agar
 Lac : lactose Man: Mannitol Glu: Glucose Suc:
 Sucrose Ox: Oxidase test Cat: Catalase test
 Cit: Citrate utilization test Mot: Motility test
 Ind: Indole test

+ : Positive test. - : Negative test. D: V ariable.

4.1.2 Kirby – Bauer disc diffusion method:

Bacterial isolates species were subjected to antibiotic sensitivity testing using different anti-microbial agents discs and the results were presented in **Table 3**

Table 3:
Susceptibility of isolated bacteria to antibiotics:

	Aantimicrobial agents											
Microorganisms	AS 20mcg	BA 25mcg	CF 30 mcg	TZP 100/10mcg	CH 30mcg	CP 5mcg	CI 30mcg	TE 30mcg	OF 5mcg	GM 10mcg	AK 30mcg	PF 10mcg
S. typhi	R	R	R	S	S	S	R	R	S	S	S	S
S. paratyphi	R	S	R	R	S	S	R	R	S	S	S	S
S.typhimurium	R	R	R	S	S	S	R	R	S	S	S	S
Sh.dysenteriae	R	R	S	S	I	S	S	R	R	S	S	S
Sh. flexenri	R	S	R	S	S	S	R	I	S	S	S	S
Sh. boydii	R	R	S	S	R	S	S	R	S	S	S	S
K. pneumonia	R	R	R	S	R	R	R	R	S	S	R	S
K. rhinoscleromatis	R	R	R	S	R	R	R	R	R	R	S	R
Proteus vulgaris	R	R	S	S	S	S	S	R	S	S	S	S
E. coli	R	R	R	R	R	R	R	S	R	S	S	R
Ps. aeruginosa	R	R	R	I	R	R	R	R	R	S	R	R

Key:

S: Susceptible

I : Intermediate

R: Resistant.

4.2 Virology:

It was found that out of the 60 fecal samples, 5 specimens contained rotavirus type A (3 %) .

Rotavirus and *Shigella boydii* were found in one of these samples (0.6%) ,rotavirus and *Pseudomonas aeruginosa* in another samples (0.6%) and remaining three samples contained *E. coli* with rotavirus (1.8%) .

Chapter five

Discussion

In developing countries like Sudan , infections are often caused via faecal-oral transmission and are thus more common in situations where sanitation is poor and water supply is contaminated and malnutrition occurs . Motarjemi *et al* (1993) noted that diarrhoea among infants and children under 5 years of age was associated with malnutrition , water supply and sanitation.

The present study was carried out to determine the prevalence of different species of enterobacteria and type A rotavirus in stools of infants aged 4 months to 5 years suffering from diarrhoea and dehydration. In faecal samples collected from 60 children with diarrhoea results showed the presence of different species of *Salmonella* mainly *S. typhi*(8.3%) , *S. paratyphi*(8.3%) , *S. typhimurium*(6.7%) and *Shigella* mainly ,*Sh. flexneri* (3.3%) , *Sh. dysenteriae* (1.7%) and *Sh. boydii*(1.7%) associated with diarrhoea in infants . In a similar study of diarrhoeal patients during the period from May to December in Hong Kong, Ling and Cheng (1993) found that , salmonellae were the most common pathogens followed by shigellae , salmonellae were isolated more often from young children aged 1-4 years whereas shigellae commonly affected young adults patients . They noted that salmonellae were more common in the hotter season months , but there was no seasonal predominance for shigellosis . In this study we found that salmonellae and shigellae were both prevalent in the summer season , but the rate of salmonellae was higher than that of shigellae in diarrhoeal patients.

In this study other organisms in less abundance included klebsiellae , *Proteus vulgaris* and *Pseudomonas aeruginosa* and were found

associated with diarrhoea . The presence of klebsiellae in acute diarrhoea was previously reported by Bernard *et al*(1980). The same authors noted *Proteus spp* frequently in normal human faeces , but often in much increased numbers in individuals receiving antibiotics therapy or during diarrhoeal diseases due to other organisms.

E. coli is a normal inhabitant of the intestinal tract . Infants less than 6 months of age are most commonly infected with enterobacteria mainly *E. coli* (Huilan, 1999) . The association of *E. coli* with diarrhoeal disease has been reported by many authors in different countries including the Sudan (Erwa, 1969) , Ruska and vesikari (1991) , Kmetova *et al* ., (1991) , Robin *et al* .,(1993) and Huilan *et al* ., (1991) .

Blank *et al*,(2000) noted that many serotypes of *E. coli* caused diarrhoea in 550 children in Sao Paulo, in 1978 and 1979; serotypes O111ab:H⁻, O111ab:H₂, and O119:H6 were significantly associated with diarrhoea in children from birth to 5 months old and were the most frequent agents of diarrhoea in this age group as compared with enterotoxigenic and enteroinvasive *E. coli* . It was not possible to serotype isolates of *E. coli* in this study.

The most common causes of diarrhoea in children are mainly viral , followed by bacteria then parasitic etiologies (Fitzgerald,1989., Cohen, 1991., Laney and Cohen, 1993) . Children 6 months to 2 years of age are more often presented with diarrhoea due to viral infections mainly rotaviruses (Huilan *et al* ., 1991) . In this study rotavirus type A was found in five (3%) of the diarrhoeal specimens . This study was conducted between May and June , but in winter month virus would have revealed in more cases. ling and ching , (1993) isolated rotavirus more frequently during the winter months .

Huilan *et al*., (1991) stated that enteropathogens are found during all seasons of year except infection with rotavirus which showed a seasonal

variation .

Mixed infection in diarrhoeic patients have been reported . Huilan *et al* , (1991) reported mixed infection of *E . coli* (EPEC) and rotavirus associated with Salmonellae , Shigellae and astrovirus.

Acquired antimicrobial resistance is a world wide problem . The situation in developing countries , however , is serious because antibiotics are taken without medical authorization or supervision .

Enteropathogenic species show variation in their susceptibility to the most antibiotics commonly used for the treatment of diarrhoea :

Blank *et al.*, (2000) found that 53% of *E. coli* strains from diarrhoeic children were resistant to ampicillin, 47% to chloramphenicol, 30% to co-trimoxazole 67% and to tetracycline. In this study all isolated *E. coli* strains were resistant to ampicillin , chloramphenicol, co-trimoxazole but sensitive to tetracycline.

Of seven shigella isolates , three were resistant to chloramphenicol and four to tetracycline Blank *et al* (2000) . In this study shigella showed susceptibility to chloramphenicol and resistance to tetracycline .

Pickering (1991) recommend ampicillin, chloramphenical, ceftriaxone, and cefotaxime for treatment of salmonella. In this study salmonellae were resistant to ampicillin and cefotaxime and sensitive to chloramphenical.

Engel and Schaeffer (1998) recommended gentamicin, amikacin and piperacillin/tazobactam for treatment of any infection caused by klebsiella, variable results were noted in this study , *K. pneumoniae* was sensitive to gentamicin , but resistant for amikacin whereas *K. rhinoscleromatis* isolates were resistant to gentamicin , but sensitive for amikacin . The two species were sensitive to piperacillin/tazobactam

Fisman and Kaye , (2000) recommend ampicillin and gentamicin for treatment of proteus , In this study , both antibiotics were effective

against proteus.

Gentamicin and ciproflaxin were recommended by Abuqaddom *et al*, (2003) for treatment of *Ps. aeruginosa*. In this study *Ps. aeruginosa* was sensitive to gentamicin, but resistant to ciproflaxin.

5.2 Conclusion :

1-In Khartoum State diarrhoeal cases in infants under 5 years of age were mainly caused by bacteria such as *E. coli* followed by *Salmonellae*, *Ps. Aeruginosa*, *Klebsiellae*, *Shigella*. was less Rotavirus and *Proteus vulgaris* were less frequent.

2-Mixed infection due to association of more than one type of organism has been noted. *E. coli* with all isolated bacteria and rotavirus and rotavirus occurred with *salmonella boydii* and *Ps. aeruginosa*.

3- Isolated bacteria showed variable susceptibility to various antibiotics in common use:

. Gentamicin was effective against infections by *Klebsiellae*, *Proteus* and *Pseudomonas*.

. Chloramphenicol was effective against *Shigellae* and *Salmonellae*.

.Tetracycline was effective against *E.coli*.

. Cefriaxone and Cefotaxime were effective against *Salmonellae*.

. Amikacin and piperacillin/ tazopactam were effective against *Klebsiellae*.

.

. Ampicillin was effective against proteus.

5-3 Recommendations:

1- Further studies are suggested to examine a large number of children with diarrhea covering different age groups during various seasons of the year.

2- Such studies should cover wider groups of bacteria and should also

include serotyping of isolates and identification of various viruses causing diarrhoea in children.

Chapter six

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